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PATENT DEPARTMENT

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DATE: 07 February 1996
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MESSAGE:

RE: WO 95/08542 KYOWA HAKKO KOGYO CO., LTD
FARNESYLTRANSFERASE INHIBITOR

Please find attached a copy of the European published patent application corresponding to the above International Application.

For your information, this application is currently with the Searching Division of the EPO and a Supplementary Search Report is expected to issue shortly. The Applicant will then be asked to confirm their intention to proceed with substantive examination of the application.

Please let me know if I can be of any further assistance.

Kind regards,



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19962
#5



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(21) Application number: 94302255.8

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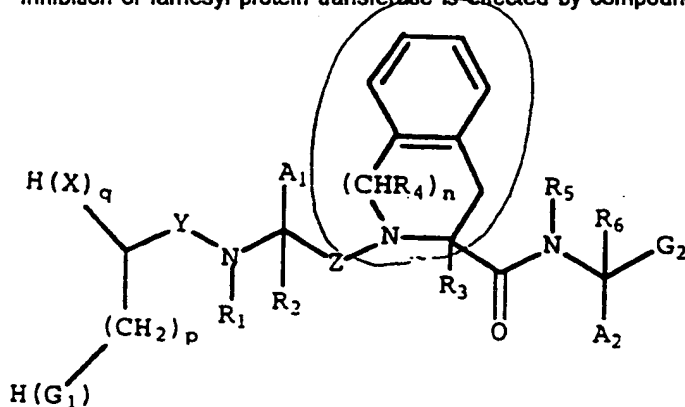
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(54) Heterocyclic inhibitors of farnesyl protein transferase.

(57) Inhibition of farnesyl protein transferase is effected by compounds of the formula



its enantiomers, diastereomers, pharmaceutically acceptable salts, prodrugs or solvates thereof, wherein:

A₁ and A₂ are each independently H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, phenyl or substituted phenyl;

G₁ is S or O;

G₂ is H, -C(O)OH, -C(O)NH₂, 5-tetrazolyl, -C(O)N(R₇)OH or -CH₂OH;

X is O or R₈N;

Y and Z are each independently -CH₂- or -C(O)-;

R₁, R₂, R₃, R₄, R₅, R₆ and R₇ are each independently H or alkyl;

R₁ may also be alkanoyl,

R₁ and A₁ taken together may be -(CH₂)_m;

R₈ is H, alkyl, phenyl, phenylalkyl, substituted phenyl, (substituted phenyl)alkyl or -C(O)R₉;

R₉ is H, alkyl, phenyl, phenylalkyl, substituted phenyl or (substituted phenyl)alkyl;

m is 3 or 4;

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(54) MEDICINAL COMPOSITION

(57) The present invention relates to an antitumor or anti-AIDS composition containing a protein-farnesyl-transferase inhibitor and an agent which decreases farnesyl pyrophosphate in vivo as active ingredients.

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(54) Title: COMBINATIONS OF INHIBITORS OF FARNESYL-PROTEIN TRANSFERASE			
(57) Abstract <p>The present invention relates to compositions comprising amounts of at least two therapeutic agents selected from a group consisting of a farnesyl protein transferase inhibitor which is an effective inhibitor of the enzyme because it is competitive with respect to the protein substrate of the enzyme and a farnesyl protein transferase inhibitor which is an effective inhibitor of the enzyme because it is competitive with respect to farnesyl pyrophosphate. Further contained in this invention are methods of inhibiting farnesyl-protein transferase and treating cancer in a mammal, which methods comprise administering to said mammal, either sequentially in any order or simultaneously, amounts of at least two therapeutic agents selected from a group consisting of a farnesyl protein transferase inhibitor which is an effective inhibitor of the enzyme because it is a competitive inhibitor with respect to the protein substrate of the enzyme and a farnesyl protein transferase inhibitor which is an effective inhibitor of the enzyme because it is a competitive inhibitor with respect to farnesyl pyrophosphate, in amounts sufficient to achieve an additive or synergistic therapeutic effect. The invention also relates to methods of preparing such compositions.</p>			

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Farnesyl transferase inhibitors cause enhanced mitotic sensitivity to taxol and epothilones

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Contributed by Samuel J. Danishefsky, December 1, 1997

ABSTRACT An important class of cellular proteins, which includes members of the p21ras family, undergoes posttranslational farnesylation, a modification required for their partition to membranes. Specific farnesyl transferase inhibitors (FTIs) have been developed that selectively inhibit the processing of these proteins. FTIs have been shown to be potent inhibitors of tumor cell growth in cell culture and in murine models and at doses that cause little toxicity to the animal. These data suggest that these drugs might be useful therapeutic agents. We now report that, when FTI is combined with some cytotoxic antineoplastic drugs, the effects on tumor cells are additive. No interference is noted. Furthermore, FTI and agents that prevent microtubule depolymerization, such as taxol or epothilones, act synergistically to inhibit cell growth. FTI causes increased sensitivity to induction of metaphase block by these agents, suggesting that a farnesylated protein may regulate the mitotic check point. The findings imply that FTI may be a useful agent for the treatment of tumors with wild-type *ras* that are sensitive to taxanes.

Potent and specific peptidomimetic inhibitors of farnesyl transferase (FTIs) have been synthesized and characterized by several laboratories (1-4). These compounds originally were conceived as potential anti-neoplastic drugs because the Ras family of proteins is farnesylated. Members of the *ras* family of protooncogenes are mutated in 30% of human cancers, and the Ras protein plays an important role in the development and progression of many human cancers. Ras is isoprenylated through the addition of a C15 farnesyl moiety. This modification confers association with the plasma membrane. Mutants of Ras that do not become membrane-associated are not transforming, and FTIs cause the reversion of transformation of fibroblasts that express the *Ha-ras* gene (reviewed in ref. 5).

FTIs also inhibit the growth of a majority of human tumor cells in culture. In a variety of animal systems, including *v-H-ras* transgenic mice and xenograft models, FTIs inhibit tumor growth, causing complete tumor regression in some murine models (6, 7). However, it is not clear that the key defarnesylated target protein is Ras. Human tumor cells without *ras* mutation often are quite sensitive to FTIs (8). The membrane association of Ki-ras and N-ras proteins is much less sensitive than is that of Ha-ras, yet tumor cells containing mutated Ki-ras can be quite sensitive to the drug (7, 9). Remarkably, even though FTI affects the processing of wild-type Ras protein, the drug has little discernible toxicity in animals at doses that have major anti-tumor effects (6).

These data do not rule out the possibility that Ras inhibition plays an important role in FTI action, but they suggest that

other targets may be involved (10). A number of other proteins are known to be farnesylated, including RhoB and Rap2, lamins A and B, phosphorylase kinase, rhodopsin kinase, cyclic GMP phosphodiesterase, and the γ subunit of transducin (5). Whatever the mechanism of inhibition of tumor cell growth, FTIs are novel drugs with wide therapeutic index in animals. Their role in the treatment of cancer patients has not been defined, but their low toxicity in animals, especially the absence of myelosuppression, suggests that they could be used effectively in combination with conventional chemotherapeutic agents. However, FTIs are cytostatic in some experimental models and could conceivably interfere with the effects of cytotoxic agents. We now have tested the effects of combinations of FTI and a variety of commonly used anti-cancer agents on human tumor cells in culture. FTI in combination with many of these agents causes potent and additive cell killing. Moreover, the effect of FTI in combination with taxol or an epothilone, agents that stabilize microtubule polymerization, is synergistic. Analysis of the mechanism of this interaction suggests that FTI enhances the mitotic block caused by exposure to these agents.

MATERIALS AND METHODS

Cell Culture and Growth Assays. MCF-7 and MDA-MB-468 breast cancer cells were obtained from the American Type Culture Collection and maintained in a 1:1 mixture of DME-to-F12 media supplemented with 100 units/ml penicillin, 100 μ g/ml streptomycin, 4 mM glutamine, and 10% heat-inactivated fetal bovine serum and incubated at 37°C at 5% CO₂. Growth assays were performed by seeding 5,000 or 10,000 cells per well in 6-well clusters and incubating for 24 h before drug treatments. Various drug treatments then were administered as outlined for individual experiments, and cells were incubated for 8-10 days, at which time they were harvested by trypsinization and counted with a Coulter counter. Doxorubicin (Pharmacia), cisplatin (Bristol-Meyers), and taxol (Bristol-Meyers) were diluted appropriately in media to achieve the desired experimental conditions. The FTI L-744832 [Merck (6)] was dissolved in PBS. desoxyepothilone A was dissolved in dimethyl sulfoxide, and appropriate dilutions were made in media to achieve desired experimental conditions. Cells were exposed to chemotherapy for 4 h to approximate *in vivo* exposure of tumors to these drugs. FTI is used in continuous culture because preclinical studies indicate tumor regrowth upon cessation of therapy (6).

Cell Cycle Analysis. Cell cycle distribution was studied in cells harvested by trypsinization, taking care to preserve the suspended and adherent cell populations. After washing in cold PBS, cell nuclei were prepared by the method of Nusse

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Abbreviations: FTI, farnesyl transferase inhibitor; FACS, flow-assisted cell sorter.

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#9

ACCELERATED COMMUNICATION

Paclitaxel (Taxol) Inhibits Protein Isoprenylation and Induces Apoptosis in PC-3 Human Prostate Cancer Cells

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SUMMARY

Paclitaxel was examined for its effects on cell survival, internucleosomal DNA fragmentation, and protein isoprenylation in the human prostate cancer cell line PC-3. Treatment of cells with paclitaxel at 5–60 nM for 24 hr resulted in a dose-dependent inhibition of cell viability (IC_{50} , 31.2 nM), which was partially prevented by supplementing the cell culture medium with two nonsterol polyisoprenyl compounds, farnesyl-pyrophosphate (-PP) and geranylgeranyl-PP (3 μ M each). Furthermore, agarose gel electrophoresis of DNA extracted from cells treated with paclitaxel (15–60 nM) for 24 hr showed DNA laddering with production of fragments of 180-base pair multiples, indicating the occurrence of apoptotic cell death. Internucleosomal DNA fragmentation by paclitaxel was also detected by a photometric enzyme immunoassay using antihistone antibodies; if culture

medium was supplemented with farnesyl-PP and geranylgeranyl-PP (3 μ M each), a reduction in mono- and oligonucleosome production was observed. The post-translational incorporation of metabolites of (RS)-[5- 3 H]mevalonolactone (100 μ Ci/ml) into prenylated proteins of PC-3 cells was inhibited by paclitaxel at 30 and 60 nM. In addition, the immunoprecipitation of p21ras and p21rap-1 proteins from PC-3 cells exposed to paclitaxel (30 and 60 nM) and labeled with (RS)-[5- 3 H]mevalonolactone showed a substantial inhibition of the incorporation of farnesyl and geranylgeranyl prenyl groups, respectively, into the aforementioned proteins. These results indicate that the inhibition of protein isoprenylation is a novel component of the complex biochemical effects of the drug and plays an important role in the mechanism of paclitaxel cytotoxicity in PC-3 cells.

Eukaryotic polypeptides that are initially synthesized with the carboxyl-terminal amino acid sequence CAAX, including a variety of signal-transducing proteins such as G proteins and cGMP phosphodiesterases, can be targeted for a series of sequential post-translational modifications (1). This novel processing pathway includes the isoprenylation of the cysteine residue with a C_{15} farnesyl or C_{20} geranylgeranyl moiety, followed by proteolysis of the three terminal residues and α -carboxyl methyl esterification of the cysteine residue (2). The isoprenoid farnesyl-PP is a particularly important intermediate in the mevalonate pathway. It is used to synthesize cholesterol (3), and it is also bound covalently to the proteins encoded by the *ras* oncogenes (4), whose mutated forms are among the most common genetic abnormalities in human cancers (5). In addition, *ras*-related, low molecular weight G proteins, including the products of the *rap-1*, *rab*, and *rho*

genes, have been shown to be geranylgeranylated (1). Thus, isoprenylation is a critical step for subcellular localization of and acquisition of biological activity by signal-transducing proteins that play a pivotal role in cell growth regulation.

Inhibitors of the enzyme HMG-CoA reductase, such as lovastatin, block the production of mevalonate and its metabolites, including farnesyl-PP and geranylgeranyl-PP, and have been shown to suppress the proliferation of many cell types (6). Inhibition of isoprenoid biosynthesis by lovastatin triggers apoptosis in the human promyelocytic cell line HL-60 (7), an effect that is also produced by paclitaxel in the same cell line (8). Paclitaxel is a terpene compound obtained from the bark of *Taxus brevifolia* and is characterized by strong affinity for tubulin protein and remarkable antitumor activity *in vitro* and *in vivo* (9). Apart from its well known antimicrotubular effect, other pharmacodynamic properties of the drug are still to be examined. In the present study, the effects of paclitaxel on apoptosis and protein prenylation were investigated in the human prostate cancer cell line PC-3.

R.D. is from the Scuola Superiore di Studi Universitari e di Perfezionamento S. Anna (Pisa, Italy). Financial support from the Italian Association for Cancer Research (Milano, Italy) to R.D. is gratefully acknowledged.

ABBREVIATIONS: PP, pyrophosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; bp, base pair(s); MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

INFORMATION DISCLOSURE
STATEMENT BY APPLICANT

(use as many sheets as necessary)

COMPLETE IF KNOWN

Sheet	1	of	2	Attorney Docket Number	19962YP
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U.S. PATENT DOCUMENTS

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FOREIGN PATENT DOCUMENTS

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No.	Foreign Patent Document			Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY
		Office	Number	Kind Code (if known)		
		EP	0 856 315 A1		Banyu Phameceutical Co., LTD.	02/20/1997
		EP	0 618 221 A2		Bristol-Myers Squibb Company	05/10/1994
		EP	0 670 314 A1		Kyowa Hakko Kogyo Co., LTD.	03/30/1995
		PCT	WO 97/17070		University of Pittsburgh	05/15/1997
		EP	0 456 180 A1		E. R. Squibb & Sons, Inc.	11/13/1991
		PCT	WO 97/01275		Banyu Phameceutical Co., LTD.	01/16/1997

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Date Considered

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(use as many sheets as necessary)</i>				COMPLETE IF KNOWN	
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				Group Art Unit	
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Sheet	2	of	2	Attorney Docket Number	19962YP

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